Claims

1. A method for randomizing polynucleotides at specific sites with no sequence related determination needed which comprises
providing at least one polynucleotide having at least one differing site and selectively randomizing the polynucleotides at or in a proximity to the at least one differing site.

2. The method of claim 1, wherein the polynucleotide is double-stranded and is derived from at least one starting single-strand polynucleotide or is a heteroduplex generated from at least two polynucleotides that differ in at least one site from each other.

3. The method of claim 1, wherein the polynucleotides or their corresponding translational products are pre-selected with respect to their genotypic and/or phenotypic features.

4. The method of claim 1, which comprises the following steps:
(a) providing polynucleotides that differ at one or more sites from each other, whereby these differing sites define start points for randomization;
(b) generating heteroduplices from these polynucleotides;
(c) recognizing resulting mismatching sites;
(d) selectively randomizing the polynucleotide at or in proximity to these mismatching sites;

5. The method of claim 4 wherein steps (a) to (d), steps (a) to (b) and/or steps (c) to (d) are carried out for multiple cycles before entering into a next step.

6. The method of claim 1, wherein the at least one differing site of the polynucleotide consists of one or more mutation(s), and the mutations comprise

(i) one or more nucleotide substitution(s),
(ii) one or more nucleotide insertion(s),
(iii) one or more nucleotide deletion(s), or
(iv) a combination of (i) to (iii).

7. The method of claim 1, which further comprises selection or screening for at least one selectively randomized polynucleotide or its corresponding translational products towards a desired property.

8. The method of claim 1, which is carried out cyclically.

9. A method for randomizing polynucleotides at specific sites which comprises the following steps:
(a) providing polynucleotides that differ at one or more sites from each other, whereby these one or more differing sites specify the sites that are to be randomized;
(b) generating heteroduplexes from the polynucleotides provided in step (a) leading to mismatches at the one or more sites;
(c) removing at least one nucleobase at one or more of the mismatches generated in step (b), by means of an agent that is able to specifically recognize mismatch sites thereby generating an abasic site at one or more mismatches;
(d) separating the heteroduplex strands from each other; and
(e) synthesizing counter strands using single strands generated as templates, thereby randomizing the polynucleotides specifically at sites where abasic sites were generated in step (c).

10. A method for randomizing polynucleotides at specific sites which comprises the following steps:
(a) providing polynucleotides that differ at one or more sites from each other, whereby these one or more differing sites specify the sites that are to be randomized;
(b) generating heteroduplices from the polynucleotides provided in step (a) leading to mismatches at the one or more sites;
(c) introducing single-strand nicks at one or more of the mismatches generated in step (b), by means of an agent that is able to specifically recognize mismatch sites;

(d) removing one or more nucleotides from the polynucleotide heteroduplex starting at the single-strand nicks generated in step (c);
(e) filling one or more gaps produced in step (d) under conditions that lead to the incorporation of one or more mismatching nucleotides, thereby randomizing the polynucleotides at the specific sites.

11. A method for randomizing polynucleotides at specific sites which comprises the following steps:
(a) providing polynucleotides that differ at one or more sites from each other, whereby these one or more differing sites specify the sites that are to be randomized;
(b) generating heteroduplices from the polynucleotides provided in step (a) leading to mismatches at the one or more differing sites;
(c) introducing single-strand nicks at one or more of the mismatches generated in step (b), by means of an agent that is able to specifically recognize mismatch sites;
(d) removing one or more nucleotides from the polynucleotide heteroduplex starting at the single-strand nicks generated in step (c);
(e) filling one or more gaps produced in step (d) at least in part with universal monomers, whereby universal monomers are characterized as being able to form basepairs alternatively with two or more of the four natural nucleobases;
(f) separating the heteroduplex strands from each other; and
(g) synthesizing counter strands using single strands generated in step (f) as templates, thereby randomizing the polynucleotides specifically at sites where universal monomers were introduced in step (e).

12. The method according to claim 10 or 11 wherein
(i) the introduction of nicks in step (c) comprises the introduction of sole single-strand break in the phosphodiester backbone at the 3' or 5' side of the mismatching site, or the removal of the entire mismatch nucleotide, or the removal of several nucleotides at or around the mismatch site; and/or
(ii) the removal of nucleotides according to step (d) is either limited to several nucleotides to generate a single-strand region in proximity to the mismatch site,

or is unrestricted to generate a gap from the mismatch position to the end of the polynucleotide; and/or

(iii) the removal of one or more nucleotides according to step (d) and with filling of the gap according to step (e) are carried out in parallel by means of a standard polymerase, a polymerase having 5'-3' exonuclease or strand displacement activity; and/or

(iv) the filling of the gap according to step (e) is carried out at least in part by use of oligonucleotides and a ligase enzyme.

13. The method according to claim 10 or 11, wherein the filling of the gap according to step (e) is carried out with a polymerase and

(i) a mixture of 3 of the 4 standard nucleotides (dATP, dTTP, dGTP, dCTG), or

(ii) separately with different compositions of mixtures of 3 nucleotides (dATP, dTTP, dGTP, dCTG), or

(iii) separately with one of the 4 standard nucleotides (dATP, dTTP, dGTP, dCTG) provided in each reaction

with optionally the separately filled gaps according to step (e) are pooled afterwards.

14. The method according to claim 10 or 11, wherein the filling of the gap according to step (e) is carried out with a polymerase under highly mutagenic conditions or with a low-fidelity polymerase having a high error rate.

15. The method according to claim 10 or 11, wherein the filling of the gap according to step (e) is carried out with a polymerase and dITP instead of dATP, dTTP, dGTP, dCTG or a mixture of dITP and dATP, dTTP, dGTP, dCTG in same or different concentrations.

16. The method of claim 1 which comprises providing variants of the polynucleotide sequence having at least one differing site and selectively randomizing the polynucleotide sequence at or in proximity to the differing site(s).

17. A method for optimizing a polynucleotide sequence with no sequence related determination needed, comprising
providing variants from this polynucleotide sequence;
randomizing the polynucleotide sequence specifically at these sites at which these variants differ from each other; and
selecting or screening a randomized pool of polynucleotides for desired properties.

18. A method for optimizing a polynucleotide towards desired properties of its translational product with no sequence related determination needed which comprises
(a) introducing stochastically random mutations into polynucleotides;
(b) selecting or screening the population of polynucleotides generated in step (a);
(c) isolating those polynucleotides which encode gene products with improved characteristics;
(d) selectively randomizing the polynucleotides at or in proximity to those site(s), at which the polynucleotides isolated in step (c) differ from each other;
(e) selecting or screening the population of polynucleotides generated in step (d)
(f) isolating those polynucleotides which encode gene products with further improved characteristics,
in the above method steps (a) to (c) and/or steps (d) to (f), and/or steps (a) to (f) are optionally repeated iteratively.